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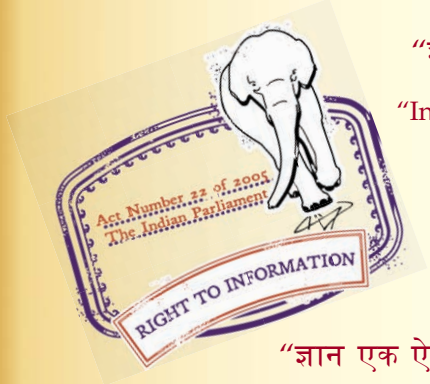

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METHODS OF SAMPLING AND TEST FOR BUTTER

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MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Indian Standard

METHODS OF SAMPLING AND TEST FOR BUTTER

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CONTENTS

Indian Standard

METHODS OF SAMPLING AND TEST FOR BUTTER

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 25 June 1966, after the draft finalized by the Dairy Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Butter is an important dairy product. Generally it is sold as either table butter or as cooking butter. This standard has been prepared with a view to providing uniform methods of analysis and facilitating the interpretation of results.

0.3 In the formulation of this standard, considerable assistance has been derived from the following publications:

Methods of sampling and testing of butterfat (ghee) and butter under Agmark, 1953. Directorate of Marketing and Inspection (Ministry of Food & Agriculture), India.

BS 769 : 1961 Methods for the chemical analysis of butter. British Standards Institution.

BS 809 : 1963 Methods for sampling milk and milk products. British Standards Institution.

FIL-IDF 12 Salt content of butter. International Dairy Federation.

Methods of analysis of the association of official agricultural chemists. 1960. Ed 9. Washington.

Standard methods for the examination of dairy products. 1960. Ed 11. American Public Health Association.

0.3.1 Full use has been made of the valuable information received from the National Dairy Research Institute, Karnal.

0.4 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard specifies the methods for sampling, and physical, chemical and bacteriological examination of butter.

2. SAMPLING

2.0 Sampling requires the most careful attention to details if the subsequent analysis is to be of value. It is, therefore, essential that a truely representative sample is drawn. But this is a difficult task and it becomes more difficult when a consignment consists of a large number of packages. However, for the purpose usual information given in the documents and certificates accompanying the consignment may be utilized to serve as a guide, and the method given in this standard should be adhered to wherever practicable. If modification is desirable, the laboratory should be consulted regarding the selection of sample.

2.1 General Requirements

2.1.1 Samples shall be drawn by an experienced person in a protected place not exposed to damp air, bright light, dust or soot. The material shall preferably be at a temperature between 0 to 15°C at the time of drawing the sample.

2.1.2 The sampling instruments (*see* **2.2**) shall be clean and dry, and shall not impart any foreign odour or flavour.

2.1.3 Samples shall be placed in clean, odourless and dry glass containers (*see* **2.3**).

2.1.4 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers from adventitious contamination.

2.1.5 While drawing the sample for bacteriological examination, all equipment and containers shall be sterile and the samples shall be collected under aseptic conditions. Equipment shall be sterilized either by heating in a hot air oven for not less than 2 hours at 160°C, or by autoclaving for not less than 15 minutes at 120°C.

2.1.6 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name and address of manufacturer, and other important particulars of the consignment.

2.1.7 Samples shall be sent to the examining laboratory as quickly as possible and shall be protected from light and contaminating odours. The samples shall be stored suitably at a temperature between 0 to 5°C. No preservative shall be added to the butter at the time of sampling.

2.1.8 Bacteriological examination shall be undertaken within 24 hours of the time of sampling.

2.2 Sampling Appliances

2.2.1 Butter triers shall be used for drawing the samples. A butter trier shall have at least 30-mm diameter and sufficient length to pass diagonally to the base of the container. The butter triers may conform to the dimensions given in Fig. 1.

2.2.2 The details of construction of the trier may be as given below.

2.2.2.1 The blade and stem of the trier should be made of stainless steel of appropriate hardness. The grip may be of stainless steel or any other suitable material which would withstand repeated sterilization.

2.2.2.2 The blade and stem may be in one piece and the transition from stem to blade shall be smooth.

2.2.2.3 The stem may be circular in cross-section and run parallel to the blade.

2.2.2.4 The blade may be tapering to the point. The degree of tapering may be less in the case of blades of triers intended for structural examination.

2.2.2.5 The grooves of the blade may have sufficient depth and the edges of the blade may be sufficiently sharp so as to facilitate the sampling of hard butter.

2.2.2.6 The surface of the blade should be well polished.

2.2.2.7 Shape, material and the finish of the trier should permit the trier to be easily cleaned and sterilized.

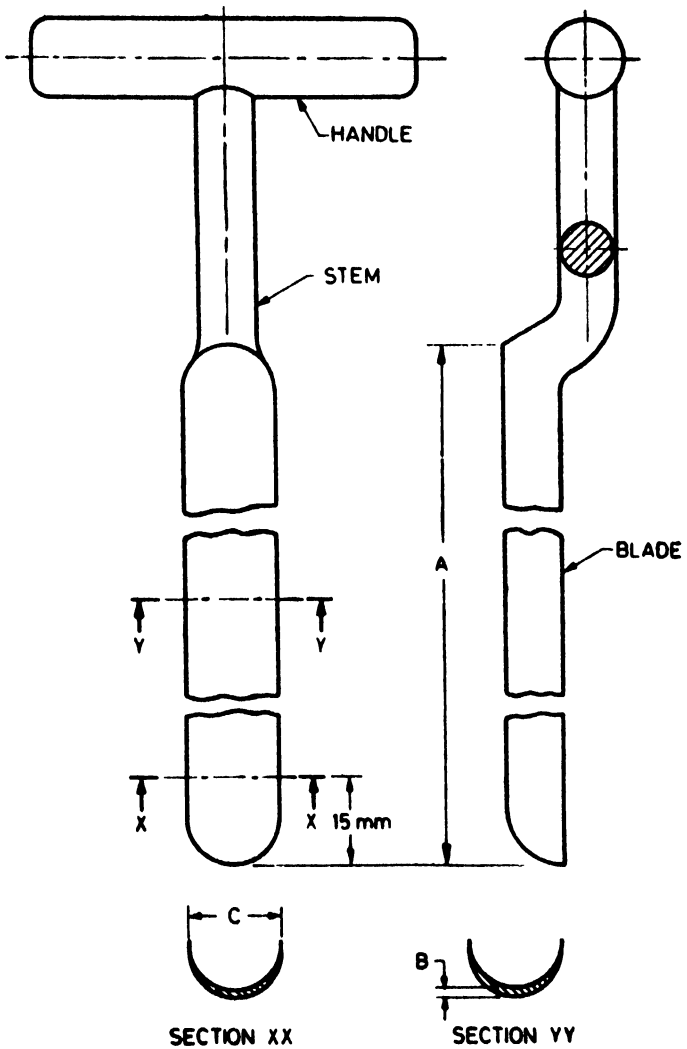
2.2.3 Spatulas and knives may be used for removing portions of samples from the triers and should be made of stainless steel.

2.3 Sample Containers

2.3.1 Wide-mouth jars and bottles of 50 and 100 ml capacity shall be used as sample containers. The approximate dimensions of the containers may be as given in Table 1.

TABLE 1 DIMENSIONS OF SAMPLE CONTAINERS

Sl. No.	Nominal Capacity (ml)	Height (mm)	Width (mm)	Width of the Mouth (mm)
i)	50	60	50	30
ii)	100	70	60	45
iii)	200-250	100	70	60



PARTICULARS	PRINCIPAL DIMENSIONS		
	Long	Medium	Short
*A**	540	265	125
B	1·8	1·5	0·5
C	17	16	11·6

*Tolerance for *A* is ± 10 percent.

All dimensions in millimetres.

FIG. 1 BUTTER TRIER

2.3.2 The jar or bottle may be closed by means of a screw cap lined with butter paper or a glass stopper. Sample containers for chemical analysis other than organoleptic tests may also be closed with rubber stoppers lined with butter paper.

2.3.3 Jars, bottles, bottle caps and stoppers should be suitable for sterilization.

2.3.4 For bacteriological examination of butter, only glass-stoppered bottles shall be used.

2.4 Scale of Sampling

2.4.1 *Lot* — All the units in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the group of units in each batch shall constitute separate lots.

2.4.2 If the butter is supplied in bulk units like casks or boxes, the number of units to be selected for sampling shall depend on the size of the lot and shall be in accordance with Table 2.

TABLE 2 NUMBER OF BULK UNITS TO BE SELECTED FOR SAMPLING

Number of Bulk Units in the Lot N	Number of Units to be Selected n
1	1
2 to 9	2
10 to 49	3
50 to 99	4
100 to 199	5
Over 200	5 for the first 200 and 1 each for 200 additional units or fraction thereof

2.4.3 If the lot is of small units like packets or tins having same batch number, the number of units selected for sampling shall be in accordance with Table 3.

TABLE 3 NUMBER OF SMALL UNITS TO BE SELECTED FOR SAMPLING

Number of Small Units in the Lot N	Number of Units to be Selected n
1 to 100	1
101 to 1 000	2
1 001 to 10 000	4
Over 10 000	4 for the first 10 000 and 1 each for 2 500 additional units or fraction thereof

2.4.4 The units to be selected for sampling shall be selected at random from the lot. To ensure randomness of selection, a random number table as agreed to between the purchaser and the vendor shall be used. In case such a table is not available, the following procedure may be adopted:

Starting from any unit in the lot, count them as 1, 2, 3,...., up to r and so on, in one order, where r is equal to the integral part of N/n, where N is the total number of units in the lot and n the number of units to be selected. Every rth unit thus counted shall be withdrawn to give the required number of the units in the sample.

2.5 Sampling Technique

2.5.1 *For Chemical Analysis*

2.5.1.1 *Hard and semi-hard butter kept under cold storage*

a) *From churns* — Four cores shall be drawn with the help of a trier at equal distances. At least two should be near the centre of the churn.

b) *From trollies* — Four cores (one each from the two ends and the other two from the sides) shall be drawn with the help of a trier.

c) *From boxes* — Three cores shall be drawn by inserting a trier vertically through the block. One core would be at the centre and the other two near diagonally opposite corners of the open end.

d) *From casks* — Three cores shall be drawn by inserting a trier at three points equidistant from the circumference of one end of the block and directed through the centre of the block.

e) *From small packets* — The samples shall consist of the unopened packets. After taking the sample for bacteriological test, the rest shall be used for chemical analysis.

2.5.1.2 *Pasty butter kept under warm conditions*

a) When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point, located 2 to 3 cm away from the ends. A suitable, clean, dry spoon, spatula or a trier should be used.

b) When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm away from the bottom and the third at an equal distance from the centre on the opposite side. At each point, draw from the deep layers three cores, roughly equidistant on the circumference. A suitable, clean, dry spoon, spatula or a trier should be used.

2.5.1.3 *Preparation of composite sample* — Taking equal amount of butter from each of the containers selected in **2.4.2** or **2.4.3**, collect about 300 g or more of the material which shall be mixed and divided into three equal parts. Each part shall be transferred to a separate sample container. One of these composite samples shall be for the purchaser, one for the vendor and the third for the referee.

2.5.2 *For Bacteriological Examination*

2.5.2.1 *Hard and semi-hard butter kept under cold storage*

a) *From churns or from butter trollies* — With a sterile or sanitized spatula or trier take a small amount of butter from not less than four different locations in the churn so that the total amount of butter is not less than 300 g. Transfer directly to a sterile or sanitized glass-stoppered bottle. Use another sterile or sanitized spatula or spoon to assist in removing the butter from the sampling instrument so as not to allow the product to come in contact with the exposed lip of the glass-stoppered bottle.

b) *From boxes and bulk packages* — With a sterile or sanitized trier bore diagonally through the container (tub or box) and remove at least two plugs with a minimum total weight of 300 g. If desired, the surface butter may be removed from the top of the plug. With the end of a sterile or sanitized spatula or spoon transfer the product to a sterile or sanitized glass-stoppered bottle, so as not to allow the product to come in contact with the exposed lip of the glass-stoppered bottle.

c) *From small retail packets* — Since there is difference in surface areas of 100, 250 and 500 g packs, remove samples from packet butter with sterile or sanitized trier in such a manner as to ensure uniformity in surface area per sample. Take 7·5 to 10 cm slice from the end of each packet and transfer it (including surface portion) with the aid of a sterile or sanitized spatula or spoon to a glass-stoppered bottle which has previously been sterilized or sanitized.

d) *Preparation of the sample* — The sample (a, b or c) shall be divided into three equal parts and placed in three sterile or sanitized glass-stoppered bottles. One sample shall be for the purchaser, another for the supplier, and the third for the referee. Close the glass-stoppered bottle and refrigerate the sample.

2.5.2.2 *Pasty butter kept under warm conditions*

a) When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point located 2 to 3 cm away from the ends, so that the total amount of butter is not less than 300 g. A sterile or sanitized spatula or trier should be used for drawing samples.

b) When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm from the bottom and the third at an equal distance from the centre on the opposite side. At each point remove a sample so that the total amount of butter is not less than 300 g. A sterile or sanitized spatula or trier should be used for drawing samples.

c) *Preparation of the sample* — Transfer directly the sample (a or b) into a sterile or sanitized glass-stoppered bottle. Use another sterile or sanitized spatula or spoon to assist in removing the butter from the sampling instrument so as not to allow the product to come in contact with the exposed lip of the glass-stoppered bottle. The sample shall be divided into three equal parts and placed in three sterile or sanitized glass-stoppered bottles. One sample shall be for the purchaser, another for the supplier and the third for the referee. Close the glass-stoppered bottles and refrigerate the sample.

2.5.2.3 *Preparation of sample for chemical analysis*

a) *Sample for analysis of butter* — Warm the sample (*see* **2.5.1**), in an air-tight container with the lid screwed down tightly or with the glass stopper, in an oven or water-bath not exceeding 39°C until by frequent vigorous shakings a homogeneous fluid emulsion (free from unsoftened pieces) is obtained at the lowest possible temperature.

b) *Sample for analysis of butterfat* — Heat a portion of emulsified butter in a beaker to a temperature of 50-60°C until the fat separates. Filter the fat layer through a dried filter paper into a dry vessel at a temperature above the solidification point of the fat, using a hot-water funnel, if necessary. Re-filter the filtrate under the same conditions, until it is clear and free from water. Liquefy the fat completely and mix before taking samples for analysis.

NOTE — Exposure to light and air of the butter sample or the butterfat obtained from it shall be as short as possible and analysis shall be carried out without delay.

3. QUALITY OF REAGENTS

3.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

*Specification for water, distilled quality (*revised*).

4. DETERMINATION OF MOISTURE

4.0 Two methods are prescribed for the determination of moisture. Method I shall be employed as routine and Method II shall be employed as the reference method.

4.1 Method I

4.1.1 *Apparatus*

4.1.1.1 *Butter-moisture tester* — with aluminium cups, tongs, riders, and 10 g weight.

4.1.1.2 *Spirit lamp or electric hot plate or gas burner*

4.1.1.3 *Desiccator* — with efficient desiccant.

4.1.2 *Procedure*

4.1.2.1 Clean the aluminium cup and dry in an oven. After cooling it to room temperature in a desiccator, properly tare the moisture scale by having the percentage riders set at zero and balancing the scale, with the empty cup without changing the position of the riders. Weigh exactly 10 g of the prepared sample in the aluminium cup, using the weight supplied with the balance.

4.1.2.2 Heat the cup over a spirit lamp, or gas burner or over an electric hot plate with constant circular motion holding the cup by means of the tongs to prevent spattering. Continue the heating of the sample, until the foaming has ceased and the curd at the bottom of the aluminium cup has attained the characteristic slightly brown (golden) colour.

NOTE — A whitish, yellowish colour indicates insufficient heating which results in low values. On the other hand a dark brown colour or black curd indicates overheating which results in high values.

4.1.2.3 Allow the cup to cool in a desiccator. When cooled, place the cup on the balance. Balance the scale and read directly the percentage of moisture. Preserve the residue for the determination of curd (**5.3.2**).

4.2 Method II

4.2.1 *Apparatus*

4.2.1.1 *Drying-oven* — maintained at 100 ± 1°C.

4.2.1.2 *Flat-bottom moisture dish* — of stainless steel, nickel, aluminium or porcelain having 7-8 cm diameter and 2·5 cm depth.

4.2.1.3 *Glass rods* — with one end flattened and about 9 cm in length.

4.2.1.4 *Desiccator* — with an efficient desiccant.

4.2.1.5 *Water-bath* — with rings to take dishes of 7·5 cm diameter.

4.2.1.6 *Clay pipe triangles*

4.2.2 *Procedure*

4.2.2.1 Clean the dish and the glass rod and dry in the oven maintained at 100 ± 1°C for at least one hour. Allow to cool to the room temperature in a desiccator and weigh the dish.

4.2.2.2 Accurately weigh into the dish 3 to 4 g of the prepared butter sample. Place the dish on a steam-bath supported on a clay pipe triangle for at least 20 minutes, stirring at frequent intervals until no moisture can be seen at the bottom of the dish. Wipe the bottom of the dish and transfer it to the oven maintained at 100 ± 1°C and keep it for 90 minutes. Allow the dish to cool in the desiccator as before and weigh. Heat the dish again in the oven for 30 minutes. Repeat the process of heating, cooling and weighing until the difference between two consecutive weights does not exceed 0·1 mg. Record the lowest weight. Preserve the residue for the determination of curd (**5.3.2**).

4.2.3 *Calculation*

4.2.3.1 Moisture, percent by weight $= \frac{100\ (W_1 - W_2)}{W_1 - W}$

where

W_1 = weight in g of the dish with the material before heating to constant weight,

W_2 = weight in g of the dish with the material after heating to constant weight, and

W = weight in g of the empty dry dish.

5. DETERMINATION OF CURD

5.0 General — Butter contains small amounts of milk proteins. The quantity varies with the method of manufacture, and the number of washings given after churning is over. In order to ensure good storage quality, butter should contain as little of the protein residue as possible. Normally protein residue does not exceed 1·5 percent. It is estimated by making fat free the residue obtained after the determination of moisture. The residue is dried and weighed.

5.1 Apparatus

5.1.1 *Gooch Crucible or Sintered Funnel* — with filter flask with adapter.

5.1.2 *Glass Funnel*

5.1.3 *Flat Bottom Flask* — 250-ml capacity.

5.1.4 *Desiccator* — with efficient desiccant.

5.1.5 *Asbestos*

5.1.6 *Glass Funnel* — with folded 12·5-cm Whatman No. 1 or its equivalent filter paper.

5.2 Reagent

5.2.1 *Petroleum Hydrocarbon Solvent* — boiling range 40-60°C.

5.3 Procedure

5.3.1 Prepare an asbestos mat in a Gooch crucible or sintered funnel, dry in the oven maintained at 100 ± 1°C, cool in the desiccator and weigh. Alternatively, dry, cool and weigh ordinary glass funnel with folded 12·5-cm filter paper.

5.3.2 Melt the residue in the moisture dish or cup from the moisture determination (**4.1.2.3** or **4.2.2.2**), add 25 to 50 ml of petroleum solvent and mix well. Fit the crucible to the filter flask or place the funnel with filter paper on a filter stand. Wet the asbestos mat or the filter paper with petroleum solvent and decant the fatty solution from the dish into the asbestos or the filter paper, leaving the sediment in the dish. Macerate the sediment twice with 20 to 25 ml of petroleum solvent and decant again the fatty solution into the asbestos or the filter paper.

5.3.3 Filter the solution and collect the filtrate in a clean, dried, tared 250-ml flat-bottom flask containing a glass bead. With the aid of a wash-bottle containing petroleum solvent, wash all the fat and sediment from the dish into the crucible or the filter paper. Finally, wash the crucible or the filter paper until free from fat, collecting all the filtrate in the flask. Preserve the filtrate for the determination of fat (**6.1.1**).

5.3.4 Dry the crucible or filter funnel in the oven maintained at 100 ± 1°C for at least 30 minutes. Cool in the desiccator and weigh. Repeat drying, cooling and weighing until the loss of weight between the consecutive weighings does not exceed 0·1 mg. Preserve the residue for the determination of salt (**7.1.2.1**).

5.4 Calculation

5.4.1 Curd and salt, percent by weight $= \frac{100\,(W_1 - W_2)}{W}$

where

W_1 = weight in g of the filter paper with residue (**5.3.4**),
W_2 = weight in g of the filter paper alone (**5.3.1**), and
W = weight in g of the sample (**4.1.2.1** or **4.2.2.2**).

5.4.2 Curd, percent by weight, is obtained by subtracting the value of salt, percent by weight (**7.1.3**) from the value obtained as in **5.4.1**.

6. DETERMINATION OF FAT

6.0 Three methods are prescribed for the determination of fat. Methods I and III shall be employed as routine methods and Method II as the reference method.

6.1 Method I

6.1.1 *Procedure* — Distil off the solution of fat in light petroleum solvent collected in a tared flask (**5.3.3**). After removing all traces of solvent, dry the flask containing fat in an oven maintained at 100 ± 1°C for one hour, cool in a desiccator and weigh. Continue the drying, cooling and weighing until the loss of weight between consecutive weighings does not exceed 0·1 mg.

6.1.2 *Calculation* — Fat, percent by weight $= \frac{100\ (W_1 - W_2)}{W}$

where

W_1 = weight in g of 250-ml flask with dried fat (**6.1.1**),

W_2 = weight in g of empty flask (**5.3.3**), and

W = weight in g of the sample (**4.1.2.1** or **4.2.2.2**).

6.2 Method II by Rose-Gottlieb Method — The fat in butter is determined using one gram of the sample by the method given in **4.2** of IS : 3509-1966*.

6.3 Method III

6.3.1 *Apparatus*

6.3.1.1 Butyrometer, 70 percent scale [*see* IS : 1223 (Part I)-1970 Specification for Apparatus for Determination of Fat by Gerber Method: Part I Butyrometers and Stoppers (*First Revision*)].

6.3.2 *Reagents*

6.3.2.1 Same as given in IS : 1224-1958 Determination of Fat in Whole Milk, Evaporated (Unsweetened) Milk, Separated Milk, Skim Milk, Buttermilk and Cream by the Gerber Method.

6.3.3 *Procedure* — Weigh 2·5 g of butter sample (**2.5.2.3**) in a 25-ml beaker and mix it with small portions of 1 : 1 sulphuric acid. Transfer the contents into the butyrometer with the help of a small funnel. Rinse the beaker about six times with small quantities of dilute sulphuric acid to make sure that all butter has been transferred. Mix 10 ml of water and 10 ml of the sulphuric acid in a small beaker. Add between 15 to 20 ml of the mixture to the butyrometer depending on its size. Add one ml of

*Methods of sampling and test for cream.

amyl alcohol. Follow the procedure specified in **5.4** to **5.9** for determination of fat in cream in IS : 1224-1958 Determination of Fat in Whole Milk, Evaporated (Unsweetened) Milk, Separated Milk, Skim Milk, Buttermilk and Cream by the Gerber Method.

7. DETERMINATION OF SALT

7.0 Two methods are prescribed for the determination of salt. Method I shall be employed as a routine and Method II as a direct method.

7.1 Method I

7.1.0 *Principle* — Salt is extracted with hot water from the dried fat-free residue obtained in moisture determination. The chlorides are precipitated by adding excess of silver nitrate. The unused silver nitrate is titrated with potassium thiocyanate, using ferric ammonium sulphate indicator.

7.1.1 *Reagents*

7.1.1.1 *Standard silver nitrate solution* — 0·05 N, standardized against standard sodium chloride. Dissolve slightly more than theoretical quantity of silver nitrate (equivalent weight 169·89) in halogen-free water and dilute to volume.

7.1.1.2 *Nitric acid* — sp gr 1·42, approximately 70 percent (*W*/*W*).

7.1.1.3 *Nitric acid* — approximately 5 N.

7.1.1.4 *Nitrobenzene*

7.1.1.5 *Ferric ammonium sulphate indicator solution* — Dissolve 50 g of ferric ammonium sulphate [$Fe_2(SO_4)_3 . (NH_4)_2 SO_4 . 24H_2O$] in 95 ml of water containing 5 ml of 5 N nitric acid.

7.1.1.6 *Standard potassium thiocyanate solution* — approximately 0·05 N, standardized against standard silver nitrate.

7.1.2 *Procedure*

7.1.2.1 Extract the salt from the residue of curd and salt (**5.3.4**) by repeated washing of the Gooch crucible or filter paper with hot water, or by placing the crucible or filter paper in a beaker of hot water. Collect the rinsings in a 100-ml measuring flask passing the solution through a filter paper. Allow to cool to room temperature and make up to volume.

7.1.2.2 Take 25 ml water extract into a 250-ml conical flask, and add an excess (normally 25 to 30 ml) of 0·05 N silver nitrate solution. Acidify with nitric acid, add 2 ml of the indicator solution and one millilitre nitrobenzene. Mix. Determine the excess of silver nitrate by titration with the potassium thiocyanate solution until the appearance of an orange tint which persists for 15 seconds.

7.1.2.3 In the same manner determine the equivalent of 25 ml or the added amount (**7.1.2.2**) of the silver nitrate as thiocyanate, using the same volumes of reagents and water.

7.1.3 *Calculation*

7.1.3.1 Sodium chloride, percent by weight $= \frac{23{\cdot}38 \times N \times (A - B)}{W}$

where

N = normality of potassium thiocyanate,

A = volume of potassium thiocyanate in the blank titration (**7.1.2.3**),

B = volume of potassium thiocyanate in the sample titration (**7.1.2.2**), and

W = weight in g of the sample (**4.1.2.1** or **4.2.2.2**).

7.2 Method II

7.2.0 *Principle* — The butter is melted in hot water and the chloride in the mixture are titrated with a solution of silver nitrate using potassium chromate as indicator.

7.2.1 *Apparatus*

7.2.1.1 *Conical flask* — 250-ml capacity.

7.2.1.2 *Burette* — 50-ml, graduated to 0·1 ml.

7.2.2 *Reagents*

7.2.2.1 *Calcium carbonate* — analytical grade, free from chloride.

7.2.2.2 *Potassium chromate indicator* — 5 percent (*w/v*) solution in water.

7.2.2.3 *Standard silver nitrate solution* — 0·1 N, standardized against standard sodium chloride. Dissolve slightly more than theoretical quantity of silver nitrate (equivalent weight 169·89) in halogen-free water and dilute to volume.

7.2.3 *Procedure* — Weigh accurately about 5 g of the sample into the 250-ml conical flask. Carefully add 100 ml of boiling distilled water. Allow to stand with occasional swirling for 5 to 10 minutes. After cooling to 50 to 55°C (titration temperature), add 2 ml of potassium chromate solution. Mix by swirling. Add about 0·25 g of calcium carbonate and mix by swirling. Titrate at 50 to 55°C with standard silver nitrate solution while swirling continuously, until the brownish colour persists for half a minute. Carry out a blank test with all the reagents in the same quantity except the sample material. The maximum deviation between duplicate determinations should not exceed 0·02 percent of NaCl.

7.2.4 *Calculation*

$$\text{Sodium chloride, percent by weight} = \frac{5{\cdot}85\,\mathcal{N}\,(V_1 - V_2)}{W}$$

where

$\mathcal{N}$ = normality of silver nitrate solution,

V_1 = volume of silver nitrate in the sample titration,

V_2 = volume of silver nitrate in the blank titration, and

W = weight in g of the sample.

8. DETERMINATION OF TITRATABLE ACIDITY

8.0 Principle — The butter is melted in hot water and the hot solution is titrated with standard alkali till neutral to phenolphthalein.

8.1 Apparatus

8.1.1 *Burette* — with soda-lime guard tube.

8.1.2 *Conical Flasks* — 250-ml capacity.

8.2 Reagents

8.2.1 *Standard Sodium Hydroxide* — 0·02 N. Prepare a concentrated stock solution of sodium hydroxide by dissolving equal parts of sodium hydroxide (sticks or pellets) in equal parts of water in a flask. Tightly stopper the flask with a rubber bung and allow any insoluble sodium carbonate to settle down for 3 to 4 days.

Use the clear supernatant liquid for preparing the standard 0·02 N solution. About 1·6 ml of stock solution is required per litre of distilled water. The solution should be accurately standardized with acid potassium phthalate (A.R.) or oxalic acid (A.R.).

8.2.2 *Phenolphthalein Indicator Solution* — Dissolve one gram of phenolphthalein in 110 ml rectified spirit (*see* IS : 323-1959*). Add 0·1 N sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 ml.

8.3 Procedure — Weigh accurately about 20 g of the butter sample in a dry 250-ml conical flask. Add 90 ml of hot, previously boiled water and shake the contents. While still hot, titrate with 0·02 N sodium hydroxide, using one millilitre of the phenolphthalein indicator.

*Specification for rectified spirit (*revised*).

8.4 Calculation

$$\text{Titratable acidity (as lactic acid), percent by weight} = \frac{9 \times N \times V}{W}$$

where

N = normality of sodium hydroxide solution,

V = volume of sodium hydroxide, and

W = weight in g of the sample.

9. DETERMINATION OF *p*H

9.0 Two methods are prescribed for the determination of *p*H. The method to be employed shall depend on the availability of the equipment.

9.1 Method I (Colorimetric Method)

9.1.1 *Apparatus*

9.1.1.1 *p*H *comparator cells with phenol red and bromocresol purple indicator disc*

9.1.1.2 *Comparator cells or uniform test-tubes in colourless glass*

9.1.1.3 *Graduated pipette* — 1-ml capacity.

9.1.2 *Reagents*

9.1.2.1 *Phenol red indicator solution* — *p*H 6·8 to 8·4.

9.1.2.2 *Bromocresol purple indicator solution* — *p*H 5·2 to 6·8.

9.1.3 *Procedure* — Weigh 12 g of the sample and warm it to 45-50°C in a water-bath. When butter melts, shake vigorously and warm again in the water-bath. After some minutes, mix the aqueous layer by alternately filling and emptying into a capillary pipette several times. Withdraw 0·5 ml of serum by means of a pipette, add to a cell of the comparator and dilute with distilled water to the 10-ml mark. Prepare a turbidity blank by diluting the serum from a second 12 g of the same butter sample. To the test solution, add phenol red solution, and compare the colour in the comparator with the phenol red comparator discs. The corrected *p*H reading for salted butter is 0·5 below the actual reading. If the *p*H is below 6·5, use bromocresol purple indicator and a bromocresol purple comparator disc.

9.2 Method II (Potentiometric Method)

9.2.1 *Apparatus*

9.2.1.1 *p*H *meter* — with glass, or quinhydrone electrode.

9.2.1.2 *Separating funnel* — 250-ml capacity.

9.2.1.3 *Beakers* — 50-ml capacity.

9.2.1.4 *Standard saturated calomel half cell* — The calomel electrode consists of a layer of purified mercury, covered with a mixture of mercury and calomel, above which is a saturated solution of potassium chloride saturated with calomel. The mixture of mercury and calomel is prepared by grinding them into a paste with a pestle and mortar. The mixture is washed with saturated potassium chloride solution by decantation several times. It is then shaken with the remainder of the potassium chloride solution. When saturated with calomel, the solution is decanted and kept in a stoppered bottle for further use.

There are many forms of electrode vessels available to set up the half cell. Connection between mercury and the potentiometer is made by a platinum wire. The two half cells are conveniently connected by an agar-agar bridge (thin glass tube, bent into a U-shape and filled with agar solution containing 5 g of agar-agar per 100 ml of saturated potassium chloride solution). The bridge shall be kept in saturated potassium chloride solution when not used.

9.2.2 *Reagents*

9.2.2.1 *Quinhydrone* (*A.R.*) — in case quinhydrone electrode is used.

9.2.2.2 *Potassium chloride* (*A.R.*) — saturated solution.

9.2.3 *Procedure*

9.2.3.1 Melt about 100 g of butter at a temperature not exceeding 50°C. Mix, transfer to a separating funnel and allow the serum to separate by gravity, maintaining the fat in the liquid condition. Run off the serum into a suitable electrode cell and mix thoroughly.

9.2.3.2 Standardize the instrument against a buffer solution of known *p*H (about 5 to 7), and check against another buffer of a different *p*H.

9.2.3.3 Use butter serum directly in the case of glass electrodes. For quinhydrone electrode, prepare butter-serum half cell by placing enough butter serum in a 25-ml beaker to cover the electrode. Add about 0·1 to 0·2 g quinhydrone and mix well. Place a clean platinum or gold electrode, rinsed with glass-distilled water and suitably supported on a stand, into the sample. The platinum electrode should preferably be immersed in boiling water, or flamed to dull red heat in a spirit-lamp, just before use.

9.2.3.4 Connect the saturated calomel and butter serum half cell to the potentiometer. Balance the potentiometer to make the reading. Equilibrium occurs almost instantaneously and no drift is experienced. Read the *p*H directly on the instrument.

10. DETERMINATION OF COPPER

10.1 Apparatus

10.1.1 *Micro-Kjeldahl Flask*

10.1.2 *Graduated Pipette* — 1-ml capacity.

10.1.3 *Measuring Flask* — 50-ml capacity.

10.1.4 *Spectrophotometer or Other Suitable Instrument* — with 435 mμ filter or wave band.

10.2 Reagents

10.2.1 *Glass Distilled Water*

10.2.2 *Nitric Acid* — sp gr 1·42.

10.2.3 *Light Petroleum* — boiling range 40-60°C.

10.2.4 *Sulphuric Acid* — 98 percent (*w/w*).

10.2.5 *Sulphuric Acid* — 5 percent solution (*v/v*).

10.2.6 *Hydrogen Peroxide* — 100 volumes.

10.2.7 *Carbon Tetrachloride*

10.2.8 *Zinc Dibenzyldithiocarbamate* — 0·05 percent (*w/v*) solution in carbon tetrachloride.

10.2.9 *Standard Copper Solution* — Dissolve 0·157 g copper sulphate pentahydrate ($CuSO_4 . 5H_2O$) in water containing 5 ml 5 percent sulphuric acid and dilute to 200 ml (1 ml = 200 μg of Cu).

10.3 Procedure

10.3.1 All glass apparatus used during this determination shall be washed with warm 5 percent (*v/v*) nitric acid and then rinsed several times with water before use.

10.3.2 Transfer 25 g of butter, accurately weighed, to a micro-Kjeldahl flask and add 1 ml of water and 4 ml of nitric acid. Place the flask in a boiling water-bath for 15 to 20 minutes shaking frequently and thoroughly. Cool to about 40°C and extract with three successive portions of light petroleum, drawing off the petroleum layer by suction or by siphon. Warm cautiously to expel the light petroleum from the aqueous layer and digest on a micro-Kjeldahl stand until the volume is about 0·5 ml. Add 0·5 ml of 98 percent sulphuric acid and a few drops of nitric acid and heat until nearly colourless. Complete the digestion and destroy and nitrosyl sulphuric acid by adding two successive amounts of 0·3 ml hydrogen peroxide, heating until white fumes are evolved after each addition.

Transfer the acid, digest to a separator with 10 ml of water and make up to about 50 ml with 5 percent sulphuric acid. Add 10 ml of the zinc dibenzylidithiocarbamate solution and shake in the stoppered funnel. Filter the lower layer through a plug of cotton wool introduced in the stem of the funnel, and then measure the optical density of the extract using a spectrophotometer at a wave length of 435 mμ or other suitable instrument with a filter giving a maximum transmission near 435 mμ. Determine the blank with the reagents under the same conditions and subtract the reading from the sample reading to obtain the optical density corresponding to the copper content in the sample.

10.3.3 For preparing a standard curve, dilute the standard copper solution to a concentration of 2 μg of copper per ml (using 5 percent solution of sulphuric acid) and transfer suitable portions to separating funnels to give a range of 0-20 μg of copper. Make up to 50 ml with 5 percent solution of sulphuric acid, develop the colour and measure the optical density exactly as for the sample. Plot a standard curve with these readings.

10.3.4 Estimate the copper content by interpolation on the standard curve and express the result in ppm.

11. DETERMINATION OF IRON

11.1 Apparatus

11.1.1 *Micro-Kjeldahl Flask*

11.1.2 *Graduated Pipette* — 1-ml capacity.

11.1.3 *Measuring Flask* — 25-ml capacity.

11.1.4 *Spectrophotometer or Other Suitable Instrument* — with 510 mμ wave band or with a filter of transmission range of 490 to 520 mμ.

11.2 Reagents

11.2.1 *Glass Distilled Water*

11.2.2 *Nitri Acid* — sp gr 1·42.

11.2.3 *Light Petroleum* — with boiling range 40-60°C.

11.2.4 *Concentrated Sulphuric Acid* — 98 percent (*w/w*).

11.2.5 *Hydrogen Peroxide* — 100 volumes.

11.2.6 *Sulphur Dioxide Solution* — 2 percent solution in water.

11.2.7 *Sodium Acetate Buffer Solution* — Dissolve 340 g sodium acetate and 160 g sodium hydroxide in one litre of water.

11.2.8 *o-Phenanthroline Solution* — Dissolve 0·25 g of 1 : 10 phenanthroline monohydrate in 20 ml hot water containing 2 to 3 drops of concentrated sulphuric acid, make up to 100 ml with water and store in a dark place. Use a freshly prepared solution.

11.2.9 *Standard Iron Solution* — Dissolve one gram of iron wire in sufficient hydrochloric acid to effect complete solution and dilute to one litre (1 ml = 1 mg of iron).

11.3 Procedure

11.3.1 All glass apparatus used during this determination shall be washed with warm 5 percent nitric acid and then rinsed several times with distilled water before use.

11.3.2 Transfer 25 g emulsified butter, accurately weighed to a micro-Kjeldahl flask, and add 1 ml of water and 4 ml of nitric acid. Place the flask in a boiling water-bath for 15 to 20 minutes shaking frequently and thoroughly. Cool to about 40°C and extract with three successive portions of light petroleum drawing off the petroleum layer by suction or by siphon. Warm cautiously to expel the light petroleum from the aqueous layer and digest on a micro-Kjeldahl stand until the volume is about 0·5 ml. Add 0·5 ml concentrated sulphuric acid and a few drops of nitric acid and heat until nearly colourless. Complete the digestion and destroy any nitrosyl sulphuric acid by adding to successive amounts of 0·3 ml of hydrogen peroxide, heating until white fumes are evolved after each addition.

11.3.3 Transfer the acid digest (a sufficient volume to contain 5 to 40 μg of iron) to a 25-ml graduated flask; dilute to 10 to 15 ml, add 1 to 2 ml of 2 percent sulphur dioxide solution and a small square of congo red paper. Titrate the mixture dropwise with sodium acetate buffer until the colour changes from blue to pink. Then add 2 ml *o*-phenanthroline solution, make up to volume and mix. Stand for at least one hour, or preferably overnight. Measure the optical density of the solution using a spectrophotometer at a wave length of 510 mμ or other suitable instrument with a filter giving a maximum transmission over the range 490-520 mμ. Determine the blank with the reagents under the same conditions and subtract the reading from the sample reading to obtain optical density corresponding to iron content in the sample.

11.3.4 For preparing a standard reference curve, dilute the standard iron solutions to a concentration of 5 μg of iron per ml, and transfer suitable portions to 25-ml graduated flask to give a range of 0-50 μg of iron. Develop the colour and measure the optical density exactly as for the sample and plot a standard curve with these readings.

11.3.5 Estimate the iron content in the sample by interpolation on the standard curve and express the result in ppm.

12. DETERMINATION OF DIACETYL AND ACETYLMETHYLCARBINOL

12.0 The diacetyl in butter is removed by distillation and estimated. In the determination of acetylmethylcarbinol, a separate sample is first treated with ferric chloride which oxidizes the acetylmethylcarbinol to diacetyl; the latter is then distilled and estimated.

12.1 Apparatus

12.1.1 *Distillation Flask* — 2-litre capacity.

12.1.2 *Beakers* — 200-ml capacity.

12.1.3 *Sintered Glass Crucibles* — with filter flask and adapter.

12.1.4 *Desiccator* — with efficient desiccant.

12.1.5 *Oven* — maintained at 120°C.

12.2 Reagents

12.2.1 *Hydroxylamine Hydrochloride* (*A.R.*) — 20 percent solution.

12.2.2 *Sodium Acetate* — 20 percent solution.

12.2.3 *Nickel Chloride* — 10 percent solution.

12.2.4 *Ferric Chloride* — 40 percent solution.

12.2.5 *Mixed Reagent* — To 4 ml of 20 percent hydroxylamine hydrochloride solution, add 4 ml of 20 percent sodium acetate solution and 2 ml of 10 percent nickel chloride solution. Filter and keep in a glass-stoppered reagent bottle protected from light. Prepare fresh every time.

12.3 Estimation of Diacetyl — Weigh 400 g of butter into a two-litre distillation flask and steam and distil into 10 ml of the freshly prepared mixed reagent. Continue steam distillation until 100 ml distillate has been collected. The glass tube at the end of the container must be submerged in the reagent. After completing the distillation, heat the distillate to 80°C, and allow to stand overnight. Filter into a weighed dried Gooch crucible. Set the filtrate aside for another 24 hours and again filter out any precipitate that may have formed. Dry the precipitate for 2 hours at 120°C with not less than 50 cm of vacuum.

Express the results as mg of nickel salt (nickeldimethyl-glyoxime) per kg of butter.

12.4 Estimation of Acetylmethylcarbinol — Add 40 ml of filtered solution of 40 percent ferric chloride to 400 g of butter in a distillation flask, and proceed as in **12.3**.

The results of this distillation will give the combined diacetyl and acetylmethylcarbinol contents. This is the preferred method for analysing butter as only one distillation is required.

To determine the amount of acetylmethylcarbinol present, subtract the results of the diacetyl determination from that for acetylmethylcarbinol. Express the results as mg of nickel salt per kg of butter.

13. DETERMINATION OF PRESUMPTIVE COLIFORM COUNT

13.0 Generally, total bacterial counts of butter cannot be used as the index of sanitary conditions of manufacture and handling since the counts may be attributed to organisms transferred from cream, to contamination derived from equipment and other sources during manufacture, or to starter bacteria frequently added to the cream for ripening. Since butter is generally prepared from pasteurized cream, presence of coliform bacteria in the product is an indication of insanitary conditions in the creamery.

13.1 Apparatus

13.1.1 *Pipettes* — sterile, delivery 10 ml and 1 ml.

13.1.2 *Petri Dishes* — sterile, 10 cm outside diameter and about 1·5 cm depth.

13.1.3 *Water-Bath* — maintained at 43 to 45°C.

13.1.4 *Incubator* — maintained at 37 ± 0·5°C.

13.1.5 *Volumetric Flask*

13.2 Reagents

13.2.1 *Preparation of Dilution Blanks* — Fill dilution bottles with phosphate buffer or Ringer's solution so that after sterilization each will contain 99 ml, or other desired amounts. Optionally use 99-ml dilution blanks, to which 11 ml of butter or 11 ml of previous dilution thereof has been added, or 9 ml blanks, to which one millilitre of butter or one millilitre of previous dilution thereof has been added in order to avoid transferring 0·1 ml quantities. After sterilization and before use, observe the amount in each blank and discard those with variations exceeding ± 2 percent. Correctly calibrated, automatic water measuring devices may also be used. When it is considered necessary to use bulk sterilized water, measure water directly into sterile dilution bottles and use prepared blanks promptly.

13.2.2 *Violet Red Bile Agar* — Prepare the medium preferably from dehydrated base (or from ingredients) consisting of 0·3 percent yeast extract, 0·7 percent peptone, 0·15 percent bile salts, one percent lactose, 0·5 percent sodium chloride, 1·5 percent agar, 0·003 percent neutral red, and 0·000 2 percent crystal violet meant for use in bacteriological work, in

water with final *p*H 7·4 ± 0·1. After complete dehydration, cool to 42 to 44°C before pouring plates. After solidification of the medium in plate, add cover layer of the medium. Preferably prepare the medium shortly before use, otherwise sterilize by autoclaving at 121°C for 15 minutes before use.

13.2.3 *Desoxycholate (Lactose) Agar* — Prepare the medium preferably from dehydrated base (or from ingredients) consisting of (a) one percent polypeptone, one percent lactose, 0·5 percent sodium chloride, 0·2 percent sodium citrate, 0·05 percent sodium desoxycholate, 1·5 percent agar, and 0·003 percent neutral red meant for use in bacteriological work in water with final *p*H 7·1 ± 0·1; or (b) one percent peptone meant for use in bacteriological work, one percent lactose, 0·1 percent sodium desoxycholate, 0·5 percent sodium chloride, 0·2 percent dipotassium phosphate, 0·2 percent ferric ammonium citrate, 1·5 percent agar, and 0·003 percent neutral red, meant for use in bacteriological work, in water, with final *p*H 7·3 ± 0·1. After complete rehydration, cool to 42 to 44°C before pouring plates. After solidification of the medium in plate, add cover layer of the medium. Preferably prepare the medium shortly before use, otherwise sterilize by autoclaving at 121°C for 15 minutes.

13.3 Procedure

13.3.1 Warm and melt the test samples of butter in the sterile container by keeping it in a water-bath maintained at 43 to 45°C for a period not exceeding 15 minutes. Agitate thoroughly so as to obtain uniform mixing of the serum, water and fat and if necessary use a sterile glass rod for complete mixing.

13.3.2 Warm the dilution blanks to about 40°C in the water-bath and sterile 10 ml and 1 ml pipettes by drawing in and forcing out the warm dilution blank 2 or 3 times. Transfer 10 ml of the melted butter into the sterile dilution blank (warmed to 40°C) in a sterile 100-ml volumetric flask and make up the volume to give a dilution of 1 : 10. Shake the dilution 25 times in the usual manner.

13.3.3 Transfer 1 ml of this dilution to a 9-ml blank in a sterile flask to obtain 1 : 100 dilution. Transfer one millilitre of the solution into sterile petri dishes in duplicate. Add to each petri dish 10 to 15 ml of violet red bile agar or desoxycholate agar previously melted and cooled to 42-44°C. Mix the contents thoroughly by gentle tilting and rotation of the plates. After the mixture has solidified, pour another layer of the same medium (5 to 6 ml) and spread evenly to cover the surface completely. When the medium has set, invert and incubate the petri dishes at 37 ± 0·05°C for 24 hours.

13.3.4 Examine the plates for presence of typical colonies of coliform bacteria indicated by dark red colonies measuring at least 0·5 mm in

diameter. Count all such colonies and report the results as number of colonies per millilitre of butter.

NOTE — Sometimes it may be necessary to use dilutions higher than 1 : 100 and more quantity of solution to get good results.

13.4 Interpretation of Results — Coliform counts exceeding 10 per ml of butter shall be taken to indicate inefficient pasteurization of cream or contamination of the product from wash water, equipment and other sources during manufacture and packaging.

14. DETERMINATION OF YEAST AND MOULD CONTENT

14.0 Total bacterial counts cannot logically be used in determining the general conditions surrounding the manufacture and handling of butter because cultures of specified organisms are frequently added to the cream and occasionally directly to the butter itself, with the result that the bacterial content of the finished butter is influenced. Yeast and mould counts of butter have, accordingly, been suggested because these micro-organisms should be present, if at all, in very small numbers.

14.1 Apparatus

14.1.1 *Sterile, Screw-Cap or Glass-Stoppered Glass Bottles* — of suitable sizes (25 ml size is convenient).

14.1.2 *Petri Dishes* — sterile, with covers (100 × 15 mm).

14.1.3 *Pipettes* — sterile, 1·1-ml.

14.1.4 *Pipettes* — sterile, 10 ml and 11 ml.

14.1.5 *Water-Bath* — maintained at 43 to 45°C.

14.1.6 *Incubator* — maintained at 25 ± 1°C.

14.1.7 *Autoclave* — for working at 121°C.

14.1.8 *pH Measuring Equipment*

14.1.9 *Buffered Water Blank* — 99 ml (sterilized).

14.2 Reagents

14.2.1 *Potato Glucose Agar (Acidified)*

Infusion from 200 g of white potatoes	1 000 ml (Boil 200 g white, peeled and sliced potatoes in about 500 ml of water for 15 minutes or until soft. Filter through cotton and make up to 1 000 ml).
Glucose	20 g
Agar	15 g

14.2.1.1 Heat the above mixture to boiling to dissolve ingredients. Distribute into tubes or flasks and autoclave for 15 minutes at 121°C. Melt in flowing steam or boiling water, cool, and acidify to *p*H 3·5 with a sterile 10 percent tartaric acid or lactic acid or citric acid solution. Mix thoroughly and pour into plates. To preserve solidifying properties of the agar, do not heat medium after the addition of the acid.

14.2.1.2 Alternately, any other growth media may be used in place of potato glucose agar, which give comparable results.

14.2.2 *Tartaric Acid, A. R.* — 10 percent solution, sterilized.

14.2.3 *Lactic Acid, A. R.* — 10 percent solution, sterilized.

14.2.4 *Citric Acid, A. R.* — 10 percent solution, sterilized.

14.2.5 *Bromphenol pH Disc and Solution* — *p*H 2·8-4·4.

14.3 Procedure

14.3.1 Warm the sample of butter contained in the sterile jar, as well as sterile buffered water blank, to about 40°C in a water-bath maintained at 42-45°C. The time required for melting the butter should not exceed 15 minutes. Agitate thoroughly so as to obtain uniform mixing of the serum, water and fat.

14.3.2 With a previously warmed sterile 10-ml pipette, transfer 10 ml of butter to a 90 ml sterile buffered water blank, which is at 37 to 40°C (11 ml of butter may be added to 99 ml of buffered water to give the same 1 to 10 dilution). Shake this dilution 25 times in the usual manner just before inoculating the petri dishes with the different dilutions given below in duplicate:

1 : 2 (5 ml of the 1 : 10 dilution); 1 : 10 (1 ml of the 1 : 10 dilution); and 1 : 100 (0·1 ml of the 1 : 10 dilution).

14.3.3 *Incubation and Colony Counting*

14.3.3.1 Prior to pouring, adjust reaction in each container (preferably electrometrical) to *p*H 3·5 ± 0·1 with sterile 10 percent tartaric or lactic or citric acid. Because remelting of acidified medium may destroy its solidifying properties, adjust only the amount needed for immediate plating. Amount of acid required for adjustment in any one flask of same batch of medium ordinarily will establish amount needed in each of the others containing equal quantities thereof.

14.3.3.2 For colorimetric adjustments, use bromophenol blue and titrate 5 ml of medium with dilute acid prepared by adding one millilitre of sterile 10 percent stock acid solution to 19 ml of water. The number of ml of dilute acid used to titrate to *p*H 3·5 will represent the amount

of stock solution that should be added to 100 ml of medium. The amount of 10 percent acid required will vary, depending upon buffering properties of the medium.

14.3.3.3 The petri dishes containing different dilutions are flooded with the melted adjusted potato dextrose agar. Not more than 30 minutes should elapse from the time of preparing dilution to the pouring of the potato dextrose agar on the plates. After solidification, the agar plates are incubated for 5 days at 21 or 25°C.

14.3.3.4 At the end of the incubation period, count the colonies of yeast and mould in the same manner as counting bacterial colonies in the plate count for milk, if interested only in the total yeast and mould count. Generally, it is desirable to differentiate between moulds and yeasts. Make a separate count of the yeast colonies, which usually will be characterized as smooth, moist, elevated or surface colonies. After counting the typical yeast colonies, count the mould colonies. Mould colonies are easily recognized by their profuse growth of hyphae.

14.3.3.5 Although the acidity of the medium is supposed to inhibit the growth of bacterial colonies, some may develop in spite of the acid. Usually these can be distinguished from the yeast colonies because they are smaller. If there is doubt regarding the identity of yeast or bacterial colonies, the colonies in question should be confirmed by microscopic observation of stained smears. Yeasts are much larger than bacteria under the microscope, and show buds.

14.3.4 *Reporting of Results* — The number of yeast and mould colonies per millilitre of butter should be reported as the total yeast and mould count, although in control work the separate yeast and mould counts are sometimes informative. To give the actual colony counts per millilitre of butter, the colony counts obtained from 1 : 2 dilution (5 ml of 1 : 10 dilution) should be multiplied by the factor 2; those from 1 : 10 dilution (1 ml of the 1 : 10 dilution) by the factor 10; and those from the 1 : 100 dilution (0·1 ml of the 1 : 10 dilution) by the factor 100.

14.3.5 *Interpretation of Results* — The following are the recommended standards:

Total Yeast and Mould Count per ml of Butter	*Sanitary Index*
Below 20	Good
21 to 50	Fair
51 to 100	Poor
Over 100	Very poor

14.3.5.1 High yeast and mould estimates in freshly churned samples indicate one or more of the following:

a) Ineffective cleaning and sterilizing, procedures,

b) Inefficient pasteurization, or

c) Carelessness in cleaning and handling equipment.

Yeast, mould and coliform counts do not accurately measure either the quality of raw materials used or the keeping qualities of butter.

15. DETECTION OF COLOURING MATTERS

15.1 Reagents

15.1.1 *Ethyl Ether*

15.1.2 *Hydrochloric Acid Solution* — 1 : 1.

15.1.3 *Sodium Hydroxide Solution* — 10 percent.

15.1.4 *Sodium Hydroxide Solution* — 2 percent.

15.1.5 *Stannous Chloride Solution* — 40 percent, containing sufficient concentrated hydrochloric acid to make the solution acidic and a small piece of tin to keep it reduced.

15.1.6 *Filter Paper*

15.2 Procedure

15.2.1 Pour about 2 g of filtered butter, dissolved in ether into each of two test tubes. To one tube add 1-2 ml of hydrochloric acid (1 : 1), and to the other about same volume of 10 percent sodium hydroxide solution. Shake the tubes well and allow them to stand. In presence of some azo dyes, acid solution shows pink to wine-red colour, while the alkaline solution in other tube shows no colour. If, on the other hand, annatto or other vegetable colour is present, alkali solution is coloured yellow, while no colour is apparent in acid solution (Red colour changing to yellow, especially on warming, in alkaline solution, may be due to presence of gallate antioxidants).

15.2.2 Pour on moistened filter paper alkaline solution of colour obtained by shaking clear butter with warm 2 percent sodium hydroxide solution. If annatto is present, paper absorbs colour, so that when washed with a gentle stream of water it remains dyed straw colour. Dry the filter, add one drop of 40 percent stannous chloride solution. Again dry carefully. If colour turns purple, presence of annatto is confirmed.

16. SCORE CARD FOR BUTTER

16.1 The score card system may be used for judging the butter for competitions, exhibitions, etc. A score card suggested for grading creamary butter is as follows:

Characteristics	*Maximum Points*	*Minimum Points*
a) Flavour (Clean, freedom from taint and rancidity)	50	40
b) Body and texture at 15 to 16°C (Firm, neither greasy nor oily, and showing granular texture on breaking)	20	15
c) Colour, appearance and finish	20	15
i) Colour (Even, that is, free from streakiness, mottling, stains or signs of curd)		
ii) Appearance & finish — Bright and clean		
d) Moisture (on pressing, the butter shall not exude beads of free moisture)	10	5
Total	100	75

16.2 The butter scoring 95 and above shall be graded as excellent, between 90-94 as very good, and between 85-89 as good, provided the individual score of each characteristic shall not be less than the minimum points mentioned against each.

16.3 Only pure products can be used for giving score card. The test shall be carried out by a small selected and trained panel of judges. A control sample of butter shall be used for comparison.

17. CHARACTERISTICS OF BUTTERFAT IN BUTTER

17.1 The characteristics of butterfat in butter shall be determined by the methods of test given in IS : 3508-1966*.

*Methods of sampling and test for ghee (butterfat).

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